Open Access

Research Article

J. Glob. Innov. Agric. Sci., 2026, 14(2):373-382
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/26.1709
http://www.jgiass.com

Effects of Biochar Supplementation in the Diet on Growth Performance, the Fecal *E. coli* and *Saccharomyces cerevisiae* counts, and Antibiotic Resistanse of *E. coli* in Growing Pigs

Nguyen Hai Quan, Phan Thi Hang, Le Duc Thao, Le Duc Ngoan and Nguyen Van Chao*

Faculty of Animal and Veterinary Sciences, University of Agriculture and Forestry, Hue University. 102 Phung Hung, Hue City 49000, Viet Nam
*Corresponding author's e-mail: nvchao@hueuni.edu.vn

The present study aimed at evaluating the effects of supplementing biochar in the diet on the growth performance, the fecal *Escherichia coli* (*E. coli*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) counts and fecal *E. coli* antibiotic resistance in growing pigs. A total 12 weaned piglets of 20.45 ± 0.59 kg (Mean ± Standard Deviation) randomly allocated to 4 treatments with 3 replicates. The animals were fed one of 4 diets including a control diet (CT), and three other diets with supplementing 0.1, 0.3 and 0.5 % of feed mainly B1, B3 and B5, respectively. The live weights and daily feed intake were recorded prior to calculate the average daily gain (ADG) and feed conversion ratio (FCR). Fecal samples were collected on day 0, 15th, 30th, 45th and 60th after supplementing biochar for *E. coli* and *S. cerevisiae* counts, and *E. coli* antibiotic resistant assessment. The results show that basal diet added up to 0.5% of biochar did not affect ADG (from 0.82 to 0.85 kg/day) and FCR (between 2.35 and 2.54). However, supplementing biochar at 3 and 5 kg/ton of feed decreased the fecal *E. coli* counts after 45 days of feeding ($p < 0.05$) but not *S. cerevisiae* ($p > 0.05$). The *E. coli* counts in the B3 (6.87 log10 CFU/g) and in the B5 (6.88 log 10 CFU/g) groups were lower than that in the CT group (7.39 log10 CFU/g) ($p < 0.05$). Supplementing biochar in diets affected on the level antibiotic resistance to AMP, CTX, CIP, CL, DO, OX in *E. coli*. The mean diameter inhibition of CTX, CL, OX against *E. coli* from B3 and B5 groups at 45th and 60th after supplemented biochar were higher than that in the CT group. Overall, dietary biochar supplementation did not affect animal performance but decreased *E. coli* counts and affected the level of antibiotic resistance to CTX, CL and OX in *E. coli*.

Keywords: Pathogenic bacteria, resistant antibiotics, cross-transmission, eukarya domain, biochar adsorption, growth performance.

INTRODUCTION

Antibiotics have been widely used in livestock production to promote growth, prevent and treat diseases (Klein et al., 2024). In fact, antibiotics are not completely absorbed in the animal body, with 30%-90% of antibiotics excreted in the animal's feces and urine (Chen et al., 2023). However, uncontrolled use of antibiotics has led to increased antibiotic resistance in many pathogenic bacteria. The consequences are reduced livestock production efficiency and public health impacts due to cross-transmission of resistant pathogens to humans. Therefore, antibiotic supplementation has been limited in the pig industry particularly because excess antibiotics in pork meat increase antibiotic resistance in human pathogens (Pandey et al., 2024). Therefore, many alternatives to antibiotics have been applied, including biochar. Several studies have reported the high prevalence of antibiotic-resistant *E. coli* isolated from pigs (Do et al., 2021; Siddi et al., 2025; Sodagari & Varga, 2023). In southern Vietnam, *E. coli* isolates showed significant resistance to chloramphenicol (85.5%), as well as tetracycline, ampicillin, amoxicillin (25.0-70.0%), trimethoprim/ sulfamethoxazole, piperacillin, enrofloxacin, marbofloxacin, gentamicin, and tobramycin. These resistance patterns were similar to those observed in *E. coli* isolates from the Mekong Delta (Duy et al., 2021), where ampicillin and trimethoprim/

Quan, N.H., Hang, P.T., Thao, L.D., Ngoan, L.D., & Chao, N.V. (2026). Effects of biochar supplementation in the diet on growth performance, the fecal E. coli and Saccharomyces cerevisiae counts, and antibiotic resistance of E. coli in growing pigs. *Journal of Global Innovations in Agricultural Sciences 14*, 373-382.
[Received 17 Jan 2025; Accepted 28 Apr 2025; Published 11 Jan 2026]

Attribution 4.0 International (CC BY 4.0)

sulfamethoxazole were among the most resistant antibiotics, while amikacin and cephalosporins showed lower resistance levels. Similar trends have been documented in Europe and Australia, where *E. coli* isolates from pigs commonly exhibit resistance to tetracycline and penicillin (Garcia-Migura et al., 2014; Smith et al., 2016). Notably, 60.0% and 26.7% of isolates were resistant to piperacillin and tobramycin, respectively—antibiotics classified as critically important for human medicine.

Saccharomyces, derived from Greek meaning "sugar mushroom," is a fungal genus within the eukarya domain that has existed for at least 400 million years. Currently, nine recognized *Saccharomyces* species are all fermentative, though research primarily focuses on *Saccharomyces cerevisiae* (*S. cerevisiae*) and *S. boulardii* (Naseeb et al., 2017). *S. cerevisiae* is one of the most extensively studied organisms in science, valued for its rapid growth and prolific carbohydrate fermentation. Previously believed to be confined to fruit-based niches, recent findings challenge this notion, revealing its presence in diverse environments, including skin, digestive tracts, milk, plants, insects, soil, tree bark, and even aquatic habitats (Goddard & Greig, 2015). Various *S. cerevisiae* strains have been utilized to enhance health, nutrition, and productivity in livestock, from cattle to poultry. Understanding microbial colonization in the digestive tract is crucial for advancing microbiome-based therapies with beneficial microorganisms (Walter et al., 2018).

Biochar is a carbon-rich material produced by pyrolysis of biomass at temperatures ranging from 350 to 1000 °C in low oxygen (EBC, 2016). Biochar can be produced from a variety of materials, including rice husks, peanut shells, coconut husks, wood, cotton fibers, and wood processing waste (Chia et al., 2015). In general, biochar has a very large internal surface area due to its extremely porous structure, which allows biochar to adsorb gases, inorganic nutrients, and dissolved organic matter (Thies & Rillig 2009). The porous structure can also serve as a habitat for bacteria and other microorganisms. Furthermore, biochar affects microbial activity by acting as an electron mediator (Sun et al., 2017) and can also alter microbial composition (Teoh et al., 2019). However, the effect on biochar supplementation in diet on pig's performance have been reported difference (Chu et al., 2013; Schubert et al., 2021). There might be dependent on the source of substrate used to produce and the procedure producing led to the different characteristics of biochar (Dung et al., 2022). Biochar is well known for its strong adsorption properties due to its ability to bind with various molecules (Chandy and Sharma, 1998). It has been demonstrated to be highly effective in removing bacteria and bacterial toxins in both in vivo and in vitro studies (Drucker et al., 1977; Gardiner et al., 1993; Naka et al., 2001). Naka et al. (2001) showed that biochar can effectively remove E. coli toxins. The adsorption and reduction of pathogenic clostridial toxins from Clostridium tetani and Clostridium botulinum using activated biochar have been studied since 1915 (Schmidt et al., 2019). Additionally, dietary supplementation with biochar effectively reduced the excretion of oocysts in pets suffering from coccidiosis or coccidial infections (Makhaleme, 2024). Studies have shown that biochar supplementation in pig feed is beneficial for a variety of reasons, including improved animal health, binding of feed contaminants (Schmidt et al., 2019), and reduction of greenhouse gases (Zhao et al., 2024). In a 10-day experiment on fattening pigs, Kim et al. (2017) showed that the number of *E. coli* in the feces of pigs fed 0.25% or 0.50% coconut biochar was significantly lower than that of the control group, while the number of *Lactobacillus* in the feces increased significantly in the biochar-supplemented groups. However, to date, there have been very limited studies on the effects of biochar on changes in the number and antibiotic resistance levels of intestinal bacteria in pigs in Vietnam.

This study was therefore, conducted to evaluate the effects of supplementing biochar in growing pig's diets on the growth performance and the number *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Escherichia coli* (*E. coli*) in feces, and antibiotic resistance of *E. coli*. From there, recommendations can be made on the supplementation of biochar to pig diets to control intestinal pathogenic bacteria and antibiotic resistance in pig farming.

MATERIALS AND METHODS

The experiment was conducted at the Veterinary Animal Husbandry Practice and Training Centre, Faculty of Animal Husbandry and Veterinary Medicine, Hue University of Agriculture and Forestry, Vietnam.

***Experimental design*:** A total of 12 weaned pigs of 20.45 ± 0.59 kg (M ± SD), two months old, kept individually in a pen were randomly assigned to 4 treatments with 3 replicates. The animals were fed one of 4 diets including the control diet (CT), and three other diets with biochar supplementation at 0.1% (B1), 0.3% (B3) and 0.5% (B5) biochar per metric ton of feed. The animals were fed twice per day at 8.00h and 16.00h. The ingredient proportion and nutritive value of the control diet are presented in Table 1.

***Feeding and biochar*:** The control diet was commercial feed bought from local animal feeding company which composed of main ingredients like maize, rice bran, soybean meal, and fishmeal. Diets were used suitable for two growing phases, 20-50 kg and 50-80 kg. The chemical composition of the control diets was presented in Table 1.

Table 1. Chemical composition of experimental feeding.

Parameters	Feeding 20-50 kg period	Feeding 50-80 kg period
Dry matter	88.70	87.90
Crude protein (%DM)	18.30	15.20

aNDF (%DM)	10.30	12.90
Ash (%DM)	5.49	5.17
ME (Kg Cal/ kg)	3150.00	3015.00
Total lysine (%DM)	1.12	0.85

Biochar production: Biochar was produced from peanut shell at the temperature of 500 °C, following the procedure described by Nguyen et al. (2018). The source of peanut shell, producing procedure and biochar characteristics were adapted from the study of Dung et al. (2022).

Measurements

***Growth performance*:** The live weight of individual pigs was weighed at the beginning and the end of the experiment; feed offered and refusals daily recorded; and the average daily gain (ADG) and feed conversion ratio (FCR) were accordingly calculated.

***Collection of fecal samples*:** Fecal samples are collected directly from the pig's rectum by wearing gloves and inserting a finger into the rectum to stimulate the collection of fecal samples. About 3-5 g of feces are collected from each pig, placed in a zip-lock plastic bag, stored in a Styrofoam box with dry ice, and then transported to the laboratory. The samples were analyzed within 3 hours of collection. Timepoint to collect the samples was set at the beginning (D0), and the day 15th (D15), 30th (D30), 45th (D45) and 60th (D60) after biochar supplementation.

***Microbiological analysis*:** The *E. coli* isolation and quantification carried out as previously described by Lupindu (2017). One gram of stool sample in 10 mL of 0.1% w/v peptone water, was stomached for 2 min. One mL portion of each homogenate was used to prepare 10-fold dilutions, to 10^5, in 0.1% w/v peptone water. Portions of 0.1 mL of the homogenate and each dilution were spread on triplicate plates of Eosin methylene blue (EMB) agar (Conda Laboratories, S.A., Spain). The typical dark to purple red colonies with metallic sheen grown on EMB agar. The plates were incubated at 37 °C for 24h, and *E. coli* colony numbers were determined from plates bearing 20 to 200 colonies. The isolates were biochemically confirmed by Gram stain, catalase, oxidase and biochemical tests (API 20E strips, Biomerieux, USA) (Hamner et al., 2007). Meanwhile, *S. cerevisiae* counts were performed as described by (Kurtzman et al., 2011). Use 1 mL (treated in peptone water as described above) diluted 10 times to 10^5 with 0.1% w/v peptone water. Use the dilution to spread (in duplicate) on yeast peptone dextrose (YPD) agar plate medium supplemented with chloramphenicol antibiotic (200 mg/L) and then incubated at 30 °C. After incubation for 48 hours, selected white colonies with round, smooth, and floating edges to be counted to determine the number of colony forming units in 1 g of sample (CFU/g). Select 10 typical colonies on each plate for identification by PCR amplification of the ITS gene region as described by Arlorio et al. (1999). A total of 5 typical colonies after being identified will be re-cultured on YPD plate agar medium and stored at 4 °C.

***Assessment of antibiotic resistance*:** The disk diffusion method was used to assess the antibiotic susceptibility of *E. coli* strains on Mueller-Hinton agar plates (Oxoid, UK) according to the guidelines and recommendations of the Clinical and Laboratory Standards Institute (CLSI 2020). A total of 10 antibiotics including ampicillin (AMP, 10 µg); cefotaxime (CTX, 30 µg); chloramphenicol (CHL, 30 µg); ciprofloxacin (CIP, 5 µg); colistin (CL, 10 µg); doxycycline (DO, 30 µg); oxacillin (OX, 1µg), gentamicin (GEN, 10 µg); streptomycin (STR, 10 µg); and tetracycline (TET, 30 µg) (Nam Khoa, Vietnam) were used for testing. Resistant, intermediate and susceptible levels were assessed according to the CLSI (2020).

***Chemical analysis*:** The DM contents of feed ingredients and a basal diet were determined by drying at 105 °C for 16 to 20 h until constant weight (Association of Official Analytical Chemists 930.15, 1990). Ash content of feed ingredients was determined by drying at 550 °C for 3 to 5 h (AOAC, 1990). Nitrogen concentration in the feed ingredients and a basal diet was determined using the automatic Kjeldahl destruction and distillation machine (Velp Scientifica, Usmate, Italia) following the Kjeldahl method (AOAC, 1990). Neutral Detergent Fibre (*aNDF*) of animal feed was analyzed including heat stable amylase and expressed inclusive of residual ash which was analyzed following the protocol of the Ankom Company, using the Ankom A200 (New York, USA).

***Statistical analysis*:** All data were managed in Microsoft Excel 2016 (MSO, 16.0.4266.1001) and analyzed using SPSS (IBM SPSS Statistics version 18.0, IBM, Armonk, NY, USA). Each pig was used as an experimental unit. *E. coli*, *S. cerevisiae* count and zone diameter (AMR of the *E. coli* and *S. cerevisiae* isolates) were analyzed by ANOVA, Tukey's test. The effect of biochar supplement on *E. coli*, *S. cerevisiae* count and the zone diameter of the inhibition against *E. coli* was determined by using the statistical model, which included the effects of treatment and the error term. The *E. coli*, *S. cerevisiae* count was not normally distributed and was $\log_{10}$ transformed before applying ANOVA. The differences were considered statistically significant at $p < 0.05$. Effect of treatments on average daily weight gain, feed intake, feed conversion, final weight was analyzed using analysis of variance (ANOVA, Tukey's test) and a post-hoc Turkey test to compare the difference between treatments when the p value of ANOVA ≤ 0.05.

RESULTS AND DISCUSSION

***Effect of biochar supplementation on growth performance*:** The performance of pigs that consumed feed inclusion different levels of biochar are presented in Table 2. There was no difference in pigs' performance including feed intake, average daily gain and feed conversion ratio, between treatments ($p > 0.05$). The results were in line with other findings. Schubert et al. (2021) reported there was no

difference of pig's performance between the control group and the group added 2% of biochar. Kupper et al. (2015) also did not find the difference on performance of pigs fed 3% biochar compared to the control group. However, Chu et al. (2013) reported a significant increase of average daily gain and feed efficiency when pigs consume feed adding 0.3% of biochar. Schmidt et al. (2019) reviewed that most of the experiments did not measure the biochar properties which is believed to have a wide range variation. In the current study, the control diet was the commercial feeding which suits the requirement of growing pigs in two measurement periods. So, there might not be any interaction between biochar and feeding ingredients which could affect animal performance. Moreover, the biochar used in the current experiment was highest at 0.5% which also might not be enough for a response on performance of pigs.

***Effect of biochar supplementation on E. coli and S. cerevisiae counts*:** The effects of biochar supplement on *E. coli* and *S. cerevisiae counts* in feces of pig are presented in Tables 3 and 4, respectively. The results in Table 2 show that supplementing biochar at 0.3% and 0.5% feed significantly decreased the *E. coli* counts on day 45th and 60th after biochar added in the diet ($p < 0.05$). The number of fecal *E. coli* tends to decline from D0 to D60 in all treatments, but not statistically different ($p > 0.05$). However, the inclusion of biochar in the diet didn't affect the *S. cerevisiae* counts at any time to collect the fecal samples (Table 4) ($p > 0.05$). Data in Table 4 also indicated that the number of *S. cerevisiae* tend to increase from D0 to D60 but not significantly ($p > 0.05$). These findings are agreement with previous reports (Naka et al., 2001; Chu et al., 2013; Kim et al., 2017). Chu et al. (2012) reported that adding 0.3% bamboo biochar to the diet for pigs increased beneficial bacteria (lactic acid bacteria) and reduced harmful bacteria (Coliform and *Salmonella* spp.). When adding activated carbon from the herbal extracts and coconut tree trunks to the diet for growing pigs, it reduced the number of *E. coli* and increase the number of *Lactobacillus* in the feces (Kim et al., 2017). In addition, activated biochar has been reported to reduce enterotoxins in many animals (Knutson et al., 2006), such as bacterial toxins (Okpabi et al., 2024) and coliform counts (Naka et al., 2001).

***Effect of biochar supplementation on anti-microbial resistant (AMR) of E. coli*:** The effects of biochar supplement on the diameter of the inhibition zone (DIZ) of AMs against isolated *E. coli* are presented in Table 5. The result in Table 5 shows that the DIZ of chloramphenicol (CHL), gentamicin (GEN), streptomycin (STR) and tetracycline (TET) weren't affected by biochar supplementation ($p > 0.05$). However, we found that the DIZ of doxycycline (DO) and TET was significantly between treatments at D0 ($p < 0.05$).

Table 2. Effect of biochar on growth performance (n = 3).

Item	Treatment#				SEM	p-value
	CT	B1	B3	B5		
Initial weight, kg	20.50	20.47	20.25	20.57	0.39	0.95
Final weight, kg	79.50	80.00	77.67	79.83	1.81	0.79
ADG, kg/day	0.84	0.85	0.82	0.85	0.02	0.76
Feed intake, kg/day	1.98	2.06	2.01	2.15	0.06	0.31
FCR	2.35	2.42	2.44	2.54	0.09	0.51

Table 3. Effect of biochar on *E. coli* counts (log10 CFU/g) in fecal samples (n = 3).

Time point##	Treatment#				SEM	p-value
	CT	B1	B3	B5		
D0	7.59	7.35	7.11	7.20	0.141	0.721
D15	7.19	7.09	7.18	7.19	0.032	0.941
D30	7.36	7.20	7.09	7.17	0.062	0.103
D45	7.39^{a}	7.11^{ab}	6.87^{b}	6.88^{b}	0.142	0.012
D60	7.13^{a}	6.99^{ab}	6.76^{b}	6.67^{b}	0.126	0.012

$^{a, b}$ Means in the same row without common letter are different at p<0.05

Table 4. Effect of biochar supplementation on *S. cerevisiae* counts (log10 CFU/g) in fecal samples (n = 3).

Time point##	Treatment#				SEM	p-value
	CT	B1	B3	B5		
D0	6.90	7.00	7.98	7.36	0.281	0.061
D15	6.69	6.85	7.94	7.25	0.322	0.061
D30	7.50	7.09	7.37	7.07	0.122	0.570
D45	7.85	7.48	7.39	7.20	0.164	0.290
D60	7.15	7.03	7.04	7.17	0.043	0.971

Table 5. Effects of biochar on the diameter of the inhibition zone of anti-microbials (AMs, mm) against the isolated *E. coli* (n: 3 replicate * 5 colonies = 15).

AMs	Time point	CT	B1	B3	B5	SEM	p-value
AMP	D0	13.9	7.1	8.1	7.3	1.85	0.481
	D15	13.3	9.3	13.3	16.9	1.79	0.631
	D30	26.1^{a}	25.7^{a}	18.9^{b}	18.5^{b}	2.42	0.001
	D45	16.6	12.2	20.0	18.3	1.94	0.632
	D60	16.4	3.6	0.0	4.7	4.10	0.062
CTX	D0	24.7	25.6	21.4	21.7	1.21	0.702
	D15	20.1	14.4	19.5	24.4	2.36	0.171
	D30	28.9^{a}	27.7^{a}	20.3^{b}	19.3^{b}	2.84	0.000
	D45	24.2^{a}	26.1^{ab}	30.7^{a}	26.2^{ab}	1.60	0.051
	D60	27.7	27.4	26.7	28.0	0.31	0.781
CHL	D0	12.3	12.7	10.8	12.0	0.48	0.550
	D15	17.3	10.9	15.3	18.4	1.90	0.542
	D30	11.7	11.5	14.1	11.5	0.72	0.312
	D45	22.1	17.5	19.0	16.7	1.38	0.491
	D60	11.9	14.7	16.6	17.7	1.47	0.181
CIP	D0	8.6	10.3	9.7	14.3	1.42	0.354
	D15	21.0	14.9	19.4	24.3	2.26	0.331
	D30	24.5^{a}	24.9^{a}	20.9^{b}	20.5^{b}	1.33	0.000
	D45	27.8	24.2	27.7	24.9	0.93	0.582
	D60	28.8	28.7	25.5	28.8	0.92	0.101
CL	D0	18.7	16.3	15.1	17.7	1.77	0.511
	D15	13.8	11.7	15.3	19.0	0.93	0.442
	D30	14.1	13.1	10.9	14.4	1.08	0.062
	D45	17.9^{a}	17.5^{a}	21.9^{b}	19.6^{ab}	1.15	0.032
	D60	20.1	20.3	20.3	20.8	0.18	0.900
DO	D0	7.4^{a}	9.1^{ab}	11.7^{ab}	17.6^{b}	2.57	0.042
	D15	13.5	11.7	12.2	15.6	1.00	0.711
	D30	15.4	13.7	13.6	15.8	0.65	0.610
	D45	15.7	15.7	16.6	14.8	0.42	0.630
	D60	15.8^{a}	13.3^{b}	14.7^{ab}	14.0^{b}	0.62	0.031
OX	D0	0.0	0.7	0.0	0.71	0.23	0.594
	D15	0.0	0.00	2.01	1.31	0.57	0.581
	D30	0.92^{a}	0.92^{a}	5.71^{b}	3.51^{ab}	1.34	0.041
	D45	0.0	0.0	1.01	1.01	0.33	0.601
	D60	8.8	5.32	0.71	2.34	2.05	0.352
GEN	D0	12.9	15.3	11.30	13.3	0.95	0.331
	D15	13.9	13.3	12.6 0	17.6	1.29	0.613
	D30	16.6	16.5	13.30	13.9	0.99	0.073
	D45	15.8	18.9	21.00	18.5	1.23	0.302
	D60	21.1	24.1	20.30	18.1	1.42	0.371
STR	D0	0.0	0.81	4.33	10.5	2.77	0.120
	D15	15.1	9.82	9.31	13.5	1.63	0.900
	D30	2.03	4.23	4.24	4.4	0.66	0.930
	D45	20.9	20.90	18.5 0	19.3	0.69	0.550
	D60	10.2	17.00	11.40	12.0	1.73	0.671
TET	D0	5.32^{a}	9.13^{ab}	12.5^{ab}	16.2^{b}	2.70	0.051
	D15	9.73	6.53	9.14	12.4	1.41	0.372
	D30	2.03	0.51	1.32	4.74	1.05	0.433
	D45	12.10	12.80	10.8 0	7.11	1.47	0.133
	D60	8.21	10.90	10.6 0	10.50	0.40	0.870

$^{a, b}$ Means in the same row without common letter are different at P<0.05

Besides those above-mentioned antibiotics, the DIZ of remained others such as ampicillin (AMP), cefotaxime (CTX), ciprofloxacin (CIP), colistin (CLI), doxycycline (DO) and oxacillin (OX) against isolated *E. coli* affected by biochar supplementation (p<0.05). However, the effects of biochar supplementation at 3 levels and 4 time points of fecal

Table 6. Effects of biochar supplementation on prevalence of AMR (number of isolated (%) of the *E. coli* isolates (n: 3 replicates*5 colonies=15).

Time point		D0				D15				D30				4				5			
Treatments		**CT**	**B1**	**B3**	**B5**	**CT**	**B1**	**B3**	**B5**	**CT**	**B1**	**B3**	**B5**	**CT**	**B1**	**B3**	**B5**	**CT[1]**	**B01[2]**	**B03[3]**	**B05[4]**
AMP	S[##]	5 (33.3)	1 (6.7)	3 (20.0)	4 (26.7)	8 (53.3)	3 (20.0)	6 (40.0)	8 (53.3)	15 (100)	15 (100)	12 (80.0)	10 (66.7)	10 (66.7)	7 (46.7)	12 (80.0)	11 (73.3)	9 (60.0)	1 (6.7)	0 (0.0)	2 (13.3)
	I	6 (40.0)	2 (13.3)	4 (26.7)	2 (13.3)	0 (0.0)	2 (13.3)	0 (0.0)	4 (26.7)	0 (0.0)	0 (0.0)	3 (20.0)	5 (33.3)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
	R	4 (26.7)	12 (80.0)	8 (53.3)	9 (60.0)	7 (46.7)	10 (66.7)	9 (60.0)	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (26.7)	7 (46.7)	2 (13.3)	3 (20.0)	4 (26.7)	14 (93.3)	15 (100)	13 (86.7)
CTX	S	5 (33.3)	4 (26.7)	1 (6.7)	6 (40.0)	2 (13.3)	0 (0.0)	3 (20.0)	6 (40.0)	9 (60.0)	10 (66.7)	0 (0.0)	0 (0.0)	3 (20.0)	6 (40.0)	14 (93.3)	8 (53.3)	8 (53.3)	11 (73.3)	10 (66.7)	10 (66.7)
	I	5 (33.3)	8 (53.3)	7 (46.7)	3 (20.0)	5 (33.3)	4 (26.7)	5 (33.3)	5 (33.3)	4 (26.7)	4 (26.7)	5 (33.3)	5 (33.3)	7 (46.7)	6 (40.0)	0 (0.0)	4 (26.7)	6 (40.0)	2 (13.3)	3 (20.0)	4 (26.7)
	R	5 (33.3)	3 (20.0)	7 (46.7)	6 (40.0)	8 (53.3)	11 (73.3)	7 (46.7)	4 (26.7)	2 (13.3)	1 (6.7)	10 (66.7)	10 (66.7)	5 (33.3)	3 (20.0)	1 (6.7)	3 (20.0)	1 (6.7)	2 (13.3)	2 (13.3)	1 (6.7)
CHL	S	0 (0.0)	0 (0.0)	1 (6.7)	1 (6.7)	8 (53.3)	3 (20.0)	6 (40.0)	7 (46.7)	0 (0.0)	0 (0.0)	5 (33.3)	2 (13.3)	10 (66.7)	5 (33.3)	8 (53.3)	5 (33.3)	0 (0.0)	2 (13.3)	7 (46.7)	7 (46.7)
	I	9 (60.0)	10 (66.7)	4 (26.7)	6 (40.0)	3 (20.0)	4 (26.7)	4 (26.7)	5 (33.3)	6 (40.0)	10 (66.7)	4 (26.7)	7 (46.7)	4 (26.7)	7 (46.7)	4 (26.7)	6 (40.0)	9 (60.0)	11 (73.3)	4 (26.7)	5 (33.3)
	R	6 (40.0)	5 (33.3)	10 (66.7)	8 (53.3)	4 (26.7)	8 (53.3)	5 (33.3)	3 (20.0)	9 (60.0)	5 (33.3)	6 (40.0)	6 (40.0)	1 (6.7)	3 (20.0)	3 (20.0)	4 (26.7)	6 (40.0)	2 (13.3)	4 (26.7)	3 (20.0)
CIP	S	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	5 (33.3)	1 (6.7)	4 (26.7)	5 (33.3)	2 (13.3)	1 (6.7)	0 (0.0)	0 (0.0)	8 (53.3)	6 (40.0)	11 (73.3)	5 (33.3)	11 (73.3)	12 (80.0)	8 (53.3)	12 (80.0)
	I	1 (6.7)	2 (13.3)	0 (0.0)	3 (20.0)	3 (20.0)	4 (26.7)	5 (33.3)	6 (40.0)	12 (80.0)	14 (93.3)	5 (33.3)	5 (33.3)	6 (40.0)	4 (26.7)	3 (20.0)	8 (53.3)	4 (26.7)	2 (13.3)	5 (33.3)	3 (20.0)
	R	14 (93.3)	13 (86.7)	15 (100)	11 (73.3)	7 (46.7)	10 (66.7)	6 (40.0)	4 (26.7)	1 (6.7)	0 (0.0)	10 (66.7)	10 (66.7)	1 (6.7)	5 (33.3)	1 (6.7)	2 (13.3)	0 (0.0)	1 (6.7)	2 (13.3)	0 (0.0)
CL	S	13 (86.7)	8 (53.3)	11 (73.3)	10 (66.7)	7 (46.7)	5 (33.3)	8 (53.3)	13 (86.7)	6 (40.0)	4 (26.7)	1 (6.7)	4 (26.7)	14 (93.3)	13 (86.7)	15 (100)	12 (80.0)	15 (100)	15 (100)	15 (100)	15 (100)
	I	0 (0.0)	5 (33.3)	1 (6.7)	2 (13.3)	1 (6.7)	1 (6.7)	2 (13.3)	0 (0.0)	4 (26.7)	4 (26.7)	4 (26.7)	6 (40.0)	0 (0.0)	1 (6.7)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	2 (13.3)	2 (13.3)	3 (20.0)	3 (20.0)	7 (46.7)	9 (60.0)	5 (33.3)	2 (13.3)	5 (33.3)	7 (46.7)	10 (66.7)	5 (33.3)	1 (6.7)	1 (6.7)	0 (0.0)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
DO	S	3 (20.0)	5 (33.3)	5 (33.3)	12 (80.0)	9 (60.0)	3 (20.0)	8 (53.3)	12 (80.0)	10 (66.7)	9 (60.0)	4 (26.7)	9 (60.0)	9 (60.0)	11 (73.3)	15 (100)	11 (73.3)	11 (73.3)	5 (33.3)	9 (60.0)	7 (46.7)
	I	5 (33.3)	6 (40.0)	7 (46.7)	2 (13.3)	2 (13.3)	8 (53.3)	2 (13.3)	2 (13.3)	5 (33.3)	5 (33.3)	10 (66.7)	6 (40.0)	5 (33.3)	4 (26.7)	0 (0.0)	4 (26.7)	4 (26.7)	9 (60.0)	5 (33.3)	8 (53.3)
	R	7 (46.7)	4 (26.7)	3 (20.0)	1 (6.7)	4 (26.7)	4 (26.7)	5 (33.3)	1 (6.7)	0 (0.0)	1 (6.7)	1 (6.7)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	1 (6.7)	0 (0.0)
OX	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (33.3)	3 (20.0)	0 (0.0)	1 (6.7)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)	3 (20.0)	2 (13.3)	0 (0.0)	0 (0.0)	1 (6.7)	1 (6.7)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)
	R	15 (100)	15 (100)	15 (100)	15 (100)	15 (100)	15 (100)	15 (100)	14 (93.3)	14 (93.3)	15 (100)	10 (66.7)	13 (86.7)	15 (100)	15 (100)	14 (93.3)	14 (93.3)	9 (60.0)	12 (80.0)	15 (100)	14 (93.3)
GEN	S	5 (33.3)	9 (60.0)	0 (0.0)	4 (26.7)	6 (40.0)	5 (33.3)	6 (40.0)	12 (80.0)	7 (46.7)	9 (60.0)	2 (13.3)	1 (6.7)	8 (53.3)	10 (66.7)	14 (93.3)	10 (66.7)	12 (80.0)	14 (93.3)	11 (73.3)	8 (53.3)
	I	6 (40.0)	3 (20.0)	6 (40.0)	4 (26.7)	4 (26.7)	5 (33.3)	3 (20.0)	3 (20.0)	8 (53.3)	6 (40.0)	12 (80.0)	14 (93.3)	5 (33.3)	5 (33.3)	1 (6.7)	4 (26.7)	2 (13.3)	1 (6.7)	4 (26.7)	2 (13.3)
	R	4 (26.7)	3 (20.0)	9 (60.0)	7 (46.7)	5 (33.3)	5 (33.3)	6 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)	2 (13.3)	0 (0.0)	0 (0.0)	1 (6.7)	1 (6.7)	0 (0.0)	0 (0.0)	5 (33.3)
STR	S	0 (0.0)	0 (0.0)	3 (20.0)	6 (40.0)	7 (46.7)	2 (13.3)	5 (33.3)	8 (53.3)	1 (6.7)	2 (13.3)	2 (13.3)	1 (6.7)	12 (80.0)	11 (73.3)	9 (60.0)	10 (66.7)	6 (40.0)	10 (66.7)	7 (46.7)	6 (40.0)
	I	0 (0.0)	1 (6.7)	0 (0.0)	2 (13.3)	1 (6.7)	6 (40.0)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	2 (13.3)	3 (20.0)	0 (0.0)	1 (6.7)	1 (6.7)	2 (13.3)	0 (0.0)	0 (0.0)	1 (6.7)	1 (6.7)
	R	15 (100)	14 (93.3)	12 (80.0)	7 (46.7)	7 (46.7)	7 (46.7)	10 (66.7)	6 (40.0)	14 (93.3)	13 (86.7)	11 (73.3)	11 (73.3)	3 (20.0)	3 (20.0)	5 (33.3)	3 (20.0)	9 (60.0)	5 (33.3)	7 (46.7)	8 (53.3)
TET	S	1 (6.7)	0 (0.0)	4 (26.7)	7 (46.7)	1 (6.7)	1 (6.7)	1 (6.7)	2 (13.3)	0 (0.0)	0 (0.0)	1 (6.7)	2 (13.3)	3 (20.0)	1 (6.7)	0 (0.0)	0 (0.0)	1 (6.7)	1 (6.7)	2 (13.3)	0 (0.0)
	I	1 (6.7)	7 (46.7)	3 (20.0)	3 (20.0)	6 (40.0)	2 (13.3)	4 (26.7)	7 (46.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)	6 (40.0)	10 (66.7)	9 (60.0)	7 (46.7)	9 (60.0)	7 (46.7)	3 (20.0)	6 (40.0)
	R	13 (86.7)	8 (53.3)	8 (53.3)	5 (33.3)	8 (53.3)	12 (80.0)	10 (66.7)	6 (40.0)	15 (100)	15 (100)	14 (93.3)	11 (73.3)	6 (40.0)	4 (26.7)	6 (40.0)	8 (53.3)	5 (33.3)	7 (46.7)	10 (66.7)	9 (60.0)

[##] S: Suscepbility; I: Intermediate; R: Resistant

collection were not so clear. We have found that the DIZ of AMP and OX in the B3 and B5 were lower than in the CL and B1 ($p < 0.05$) at D30. At D30 and D45, the DIZ of CTX and CIP was also lower in B3 and B5 than in CL and B1.

At D45, the DIZ of CLI was lower in the CL and B1 than in the B3 ($p < 0.05$) and not different in the B5 ($p > 0.05$). In addition, the DIZ of DO in B1 and B5 were lower than in the CL ($p < 0.05$) but consistent with the B3 ($p > 0.05$) at D60.

The affected of biochar supplement on the prevalence of AMR of isolated *E. coli* is presented in Table 6. Overall, the isolated *E. coli* exhibited high resistance to OX, STR, and TET, and susceptibility to CLI, and GEN. The frequencies of

resistance to CIP, CLI, DO, and GEN in all groups decreased from first to last days of the experiment. The frequencies of susceptibility to most of AM were increased from first to last day of the experiment.

In this study, the effect of biochar supplementation in the diet of growing pigs on the resistance level of some antibiotics (AMP, CTX, CIP, CLI, OX) of *E. coli* was found. The underlying mechanisms of resistance gene reduction by biochar have not been fully elucidated, with studies suggesting that factors such as raw materials used for production (Yang et al., 2022), size (Tong et al., 2023), and dosage (Zhou et al., 2023) play an important role. In addition, significant differences in the levels of antibiotic resistance in the gut microbiota of animals treated with and without antibiotics during different growth stages have been reported (Burow et al., 2019).

Furthermore, high rates (small the DIZ) of some antibiotics such as DO and TET against *E. coli* were observed at the beginning of the experiment. These findings suggest that the pigs were more likely to carry AMR *E. coli*, which could come from the sows or the rearing environment (Lim et al., 2023). Transmission of resistant bacteria from the sows to their piglets has been reported by Mathew et al. (2005). *E. coli* from piglets were more likely to be resistant to AMP or azithromycin if bacteria isolated from their respective mothers were also resistant to these agents (Burow et al., 2019). This suggests vertical transmission of AMR bacteria, such as *E. coli*, from mother to piglet. Burow et al. (2019) also reported that AMP and TET resistance was prevalent before the use of these AMs for treatment and that AMP and TET resistance was prevalent in *E. coli* isolated from pigs not treated with beta-lactams and TET. To date, there have been very limited studies on the effects of biochar on changes in antibiotic resistance levels of the intestinal bacteria. The results of this study may suggest a useful solution in controlling antibiotic resistance in the intestinal bacteria.

***Conclusion*:** Animal performance was not affected by adding biochar in the diets. The biochar supplementation in the growing pig's diet reduced the number of *E. coli* without affecting the number of *S. cerevisiae*. Moreover, biochar also affected the antibiotic resistance of fecal *E. coli*. In recommendation, biochar can be included 0.3-0.5% of pig feed to reduce *E. coli* and the resistance level of some antibiotics of *E. coli*.

***CRediT author statement*:** All authors contributed to the study's conception and design. Nguyen Hai Quan developed the original hypotheses, designed the experiments, and collaborated in interpreting the results; Le Duc Thao, Phan Thi Hang and Nguyen Van Chao collected the data for this study, conducted the statistical analyses, collaborated in the interpretation of the results; Nguyen Hai Quan, Le Duc Thao, Le Duc Ngoan and Nguyen Van Chao collaborated in interpreting the results, and finalized the manuscript. All authors have read and approved the finalized manuscript.

***Conflict of interest*:** The authors clarify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript

***Funding*:** The authors acknowledged the International Foundation for Science (IFS) for its partly funding under grant code B-6206-2.

***Ethical statement*:** The current experiment mainly works on animal feces in the laboratory and permission from bioethics committee was sorted out.

***Availability of data and material*:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

***Acknowledgment*:** The authors acknowledged the International Foundation for Science (IFS) for its partly funding under grant code B-6206-2, the graduate students of the Faculty of Animal and Veterinary Sciences for taking care the animals and running at the laboratory.

***Informed consent*:** N/A

***Consent for publication*:** All authors submitted consent to publish this research article in JGIAS.

***SDGs addressed*:** Good health and well-being, Zero Hunger, Responsible Consumption and Production.

***Policy referred*:** The global and national policy on reducing or banning antibiotic use as growth promoters in livestock feed; Policy Background (as reflected in the text); World Health Organization (WHO); Food and Agriculture Organization (FAO); World Organisation for Animal Health (WOAH, formerly OIE).

***Publisher's note*:** All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Arlorio, M., Coïsson, J. D., & Martelli, A. (1999). Identification of *Saccharomyces cerevisiae* in bakery products by PCR amplification of the ITS region of ribosomal DNA. *European Food Research and Technology, 209*, 185-191.

Association of Official Analytical Chemists. (1990). *Official methods of analysis* (15th ed.). Arlington, VA. (Method 942.05)

Burow, E., Rostalski, A., Harlizius, J., Gangl, A., Simoneit, C., Grobbel, M., Kollas, C., Tenhagen, B.-A., & Käsbohrer, A. (2019). Antibiotic resistance in *Escherichia coli* from pigs from birth to slaughter and its association with antibiotic treatment. Preventive Veterinary Medicine, 165, 52-62.

Chandy, T., & Sharma, C. P. (1998). Activated charcoal microcapsules and their applications. *Journal of Biomaterials Applications, 13*, 128-157.

Chen, J., Wu, Y., & Cheng, D. (2023). Dynamics of oxytetracycline, sulfamerazine, and ciprofloxacin and related antibiotic resistance genes during sewage sludge composting. *Solid Waste, 2023*, 272.

Chia, C. H., Downie, A., & Munroe, P. (2015). Characteristics of biochar: Physical and structural properties. In *Biochar for environmental management* (pp. 89-109). Routledge.

Chu, G., Kim, J., Kim, H., Ha, J., Jung, M., Song, Y., Cho, J., Lee, S., Ibrahim, R., Lee, S. S., & Song, Y. (2013). Effects of bamboo charcoal on growth performance, blood characteristics and noxious gas emission in fattening pigs. *Journal of Applied Animal Research, 41*, 48-55.

Chu, G. M., Jung, C. K., Kim, H. Y., Ha, J. H., Kim, J. H., Jung, M. S., Lee, S. J., Song, Y., Ibrahim, R. I., Cho, J. H., Lee, S. S., & Song, Y. M. (2012). Effects of bamboo charcoal and bamboo vinegar as antibiotic alternatives on growth performance, immune responses and fecal microflora population in fattening pigs. *Animal Science Journal, 84*, 113-120.

Clinical and Laboratory Standards Institute. (2020). *Performance standards for antimicrobial susceptibility testing* (26th ed.). Clinical and Laboratory Standards Institute.

Do, D., Anh, N., Thoa, N., Le, P. K. T., S. C. M. J. H., Nam, N., & Nguyen, T. (2021). Identification of genotype and phenotype of antimicrobial resistance of *Escherichia coli* isolates from pigs in southern Vietnam. *Thai Journal of Veterinary Medicine, 51*, 125-132.

Dung, D. V., Phung, L. D., Ngoan, L. D., Quan, N. H., Tra, T. T. T., Tam, V. T. M., Ba, N. X., Thao, L. D., & Roubisk, H. (2022). Effects of biochar produced from tropical rice husk and peanut shell at different processing temperatures on in vitro rumen fermentation and methane production. *Journal of Animal and Plant Sciences, 32*, 631-637.

Drucker, M., Goldhar, J., Ogra, P., & Neter, E. (1977). The effect of attapulgite and charcoal on enterotoxicity of *Vibrio cholerae* and *Escherichia coli* enterotoxins in rabbits. *Infection, 5*, 211-213.

Duy, D. T., Anh, N. L. L., Thoa, N. T. K., Phitsanuwattana, K., Thongratsakul, S., Carrique-Mas, J., Hien, L., Nam, N. T. T., & Toan, N. T. (2021). Identification of genotype and phenotype of antimicrobial resistance of *Escherichia coli* isolates from pigs in southern Vietnam. *Thai Journal of Veterinary Medicine, 51*, 125-132.

European Biochar Foundation (EBC). (2012). *European Biochar Certificate—Guidelines for a sustainable production of biochar* (Version 4.7, 18 October 2013). http://www.european-biochar.org/en/download

Garcia-Migura, L., Hendriksen, R. S., Fraile, L., & Aarestrup, F. M. (2014). Antimicrobial resistance of zoonotic and commensal bacteria in Europe: The missing link between consumption and resistance in veterinary medicine. *Veterinary Microbiology, 170*, 1-9.

Gardiner, K., Anderson, N., McCaigue, M., Erwin, P., Halliday, M., & Rowlands, B. (1993). Adsorbents as antiendotoxin agents in experimental colitis. *Gut, 34*, 51-55.

Goddard, M. R., & Greig, D. (2015). *Saccharomyces cerevisiae*: A nomadic yeast with no niche? *FEMS Yeast Research, 15*, fov009.

Hamner, S., Broadaway, S. C., Mishra, V. B., Tripathi, A., Mishra, R. K., Pulcini, E., Pyle, B. H., & Ford, T. E. (2007). Isolation of potentially pathogenic *Escherichia coli* O157:H7 from the Ganges River. *Applied and Environmental Microbiology, 73*, 2369-2372.

Kim, K. S., Kim, Y.-H., Park, J.-C., Yun, W., Jang, K.-I., Yoo, D.-I., Lee, D.-H., Kim, B.-G., & Cho, J.-H. (2017). Effect of organic medicinal charcoal supplementation in finishing pig diets. *Korean Journal of Agricultural Science, 44*, 50-59.

Klein, E. Y., Impalli, I., Poleon, S., Denoel, P., Cipriano, M., Van Boeckel, T. P., Pecetta, S., Bloom, D. E., & Nandi, A. (2024). Global trends in antibiotic consumption during 2016-2023 and future projections through 2030. *Proceedings of the National Academy of Sciences, 121*, e2411919121.

Knutson, H. J., Carr, M. A., Branham, L. A., Scott, C. B., & Callaway, T. R. (2006). Effects of activated charcoal on binding *E. coli* O157:H7 and *Salmonella typhimurium* in sheep. *Small Ruminant Research, 65*, 101-105.

Kupper, T., Fischlin, I., Häni, C., & Spring, P. (2015). Use of a feed additive based on biochar for mitigation of ammonia emissions from weaned piglets and broilers. In I. Körner (Ed.), *RAMIRAN 2015—16th International Conference Rural-Urban Symbiosis*. TuTech Verlag, Hamburg.

Kurtzman, C. P., Fell, J. W., Boekhout, T., & Robert, V. (2011). Methods for isolation, phenotypic characterization and maintenance of yeasts. In *The yeasts* (5th ed., pp. 87-110). Elsevier, London.

Lim, J. A., Cha, J., Choi, S., Kim, J. H., & Kim, D. (2023). Early colonization of the intestinal microbiome of neonatal piglets is influenced by the maternal microbiome. *Animals (Basel), 13*, 3378.

Lupindu, A. M. (2017). Isolation and characterization of *Escherichia coli* from animals, humans, and environment. In A. Samie (Ed.), *Escherichia coli: Recent advances on physiology, pathogenesis and biotechnological applications*. IntechOpen.

Makhaleme, M. (2024). The response of layers to dietary supplementation with activated charcoal (Master's thesis). National University of Lesotho, Faculty of Agriculture.

Mathew, A., Garner, K., Ebner, P., Saxton, A., Clift, R., & Liamthong, S. (2005). Effects of antibiotic use in sows on resistance of *E. coli* and *Salmonella enterica* Typhimurium in their offspring. *Foodborne Pathogens and Disease, 2*, 212-220.

Naka, K., Watarai, S., Inoue, K., Kodama, Y., Oguma, K., Yasuda, T., & Kodama, H. (2001). Adsorption effect of activated charcoal on enterohemorrhagic *Escherichia coli*. *Journal of Veterinary Medical Science, 63*, 281-285.

Naseeb, S., James, S. A., Alsammar, H., Michaels, C. J., Gini, B., Nueno-Palop, C., Bond, C. J., McGhie, H., Roberts, I. N., & Delneri, D. (2017). *Saccharomyces jurei* sp. nov., isolation and genetic identification of a novel yeast species from *Quercus robur*. *International Journal of Systematic and Evolutionary Microbiology, 67*, 2046-2052.

Nguyen, X. L., Do, P. T. M., Nguyen, C. H., Kose, R., Okayama, T., Pham, T. N., Nguyen, P. F., & Miyanishi, T. (2018). Properties of biochars prepared from local biomass in the Mekong Delta, Vietnam. *BioResources, 13*, 7325-7344.

Okpabi, T. S., Lawson, S., Gift, W., & Ogbonda, K. (2024). Detoxification of bacterial toxins in soured soups using activated charcoal. *International Journal of Agriculture and Animal Production, 4*, 9-18.

Pandey, S., Doo, H., Keum, G. B., Kim, E. S., Kwak, J., Ryu, S., Choi, Y., Kang, J., Kim, S., & Lee, N. R. (2024). Antibiotic resistance in livestock, environment and humans: One health perspective. *Journal of Animal Science and Technology, 66*, 266-278.

Schubert, D. C., Chuppava, B., Witte, F., Terjung, N., & Visscher, C. (2021). Effect of two different biochars as a component of compound feed on nutrient digestibility and performance parameters in growing pigs. *Frontiers in Animal Science, 2*, 633958.

Schmidt, H.-P., Hagemann, N., Draper, K., & Kammann, C. (2019). The use of biochar in animal feeding. *PeerJ, 7*, e7373.

Siddi, G., Piras, F., Meloni, M. P., Migoni, M., Gymoese, P., Fredriksson-Ahomaa, M., De Santis, E. P. L., & Scarano, C. (2025). Characterization and antimicrobial resistance of commensal and pathogenic *Escherichia coli* from pigs in Sardinia (Italy). *International Journal of Food Microbiology, 111156*.

Smith, M., Jordan, D., Gibson, J., Cobbold, R., Chapman, T., Abraham, S., & Trott, D. (2016). Phenotypic and genotypic profiling of antimicrobial resistance in enteric *Escherichia coli* communities isolated from finisher pigs in Australia. *Australian Veterinary Journal, 94*, 371-376.

Sodagari, H. R., & Varga, C. (2023). Evaluating antimicrobial resistance trends in commensal *Escherichia coli* isolated from cecal samples of swine at slaughter in the United States, 2013-2019. *Microorganisms, 11*, 1033.

Sun, T., Levin, B., Guzman, J., Enders, A., Muller, D., Angenent, L., & Lehmann, J. (2017). Rapid electron transfer by the carbon matrix in natural pyrogenic carbon. *Nature Communications, 8*, 14873.

Teoh, R., Caro, E., Holman, D. B., Joseph, S., Meale, S. J., & Chaves, A. V. (2019). Effects of hardwood biochar on methane production, fermentation characteristics, and the rumen microbiota using rumen simulation. *Frontiers in Microbiology, 10*, 1534.

Thies, J., & Rillig, M. (2009). Characteristics of biochar: Biological properties. In J. Lehmann & S. Joseph (Eds.), *Biochar for Environmental Management* (pp. 85-105). Earthscan Publications Ltd.

Tong, Z., Liu, F., Sun, B., Tian, Y., Zhang, J., Duan, J., Bi, W., Qin, J., & Xu, S. (2023). Effect of biochars with different particle sizes on fates of antibiotics and antibiotic resistance genes during composting of swine manure. *Bioresource Technology, 370*, 128542.

Walter, J., Maldonado-Gómez, M. X., & Martínez, I. (2018). To engraft or not to engraft: An ecological framework for gut microbiome modulation with live microbes. *Current Opinion in Biotechnology, 49*, 129-139.

Yang, S., Wen, Q., & Chen, Z. (2022). Biochar induced inhibitory effects on intracellular and extracellular antibiotic resistance genes in anaerobic digestion of swine manure. *Environmental Research, 212*, 113530.

Zhao, Y., Jiang, H., Gao, J., Wan, X., Yan, B., Liu, Y., Cheng, G., Chen, L., & Zhang, W. (2024). Effects of biochar application methods on greenhouse gas emission and nitrogen use efficiency in paddy fields. *Science of the Total Environment, 915*, 169809.

Zhou, Y., Leong, S. Y., & Li, Q. (2023). Modified biochar for removal of antibiotics and antibiotic resistance genes in the aqueous environment: A review. *Journal of Water Process Engineering*, *55*, 104222.